(f) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

233. (New) The vector construct of claim 157 wherein when said vector construct is integrated by non-homologous recombination into the genome of a eukaryotic host cell in such a way that an endogenous gene in said genome is transcriptionally activated, then said positive selectable marker is expressed in active form and said negative selectable marker is either not expressed or is expressed in inactive form.--

REMARKS

I. Status of the Claims

Claims 1-7, 10-15, 20-36, 60-63, 70, 75, 83, 84, 94, 95, 124-127, 130, 133-156, 158, 160, 163, 168, 176, 184-222, and 227-231 have been cancelled without prejudice to or disclaimer of the subject matter contained therein. Claims 64, 69, 76, 77, 81, 89, 99, 104, 107, 108, 128, 129, 159, 161, 162, 165, 166, 173, 174, 180, and 181 have been amended to reflect changes in dependency following cancellation of claims. New claim 232 has been added. This claim combines steps (a)-(e) in claim 87 with a step that is directed to introducing the isolated and cloned cell into an animal. Support for this claim can be found in the specification, *inter alia*, on pages 36, 51 and 52. New claim 233 has been added. This claim specifies that the vector construct, when integrated by non-homologous recombination, expresses the positive selectable marker in active form and the negative selectable marker is either expressed in inactive form or not expressed. Support for this amendment can be found in the specification, *inter alia*, in Figures 9 and 10 and on pages 18-20.

No new matter is added by way of claim amendment. Claims 58, 59, 64-69, 71-74, 76-82, 85-93,96-123, 128, 129, 131-132, 157, 159, 161, 162, 164-167, 169-175, 177-183, 223-226, 232,

and 233 are pending in the present application. The Examiner's comments in the Office Action are addressed below in the order set forth therein. Applicants respectfully request reexamination and reconsideration of the application following entry of the amendments and in view of the remarks below.

II. The Rejections

A. The Rejection Under 35 U.S.C. § 112, First Paragraph

On page 2 of the Office Action, claims 73, 77-80, 82, 85-93, 98-106, 108-123, 128-132, 180-183, 223-224, and 226 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that they are not enabled. Applicants respectfully traverse the rejection.

The Examiner has taken the position, based on disclosed exemplary experiments, that an undue burden of experimentation would have been required to activate a broad range of genes. The rationale for the rejection is presented on pages 2-5 of the Office Action. First, the Examiner states that there is "no evidence that all the claimed endogenous genes would have been activated by the claimed methods and would have yielded increased production of proteins from the endogenous genes listed." He points out that the specification shows examples of activating transmembrane protein coding sequences or novel DNA sequences, but not growth factors, cytokines, hormones, etc. (page 4). The Examiner then concludes that the probability of activating any other listed genes is zero because none of these genes is activated in the exemplary experiments.

Further, on page 4 of the Office Action the Examiner asserts that there is no evidence of increased expression, even of the transmembrane protein that was activated. Further, the Examiner questions the functionality of the activated protein and whether activation would have been maintained in cells *in vivo*.

Finally, the Examiner asserts that "there is no way of finding out which gene has been activated" (page 4). Thus, the Examiner questions how a given activated gene can be identified using routine experimentation.

Essentially, the Examiner argues that the experiments in the specification provide evidence that a broad range of genes can not be activated using the disclosed vectors and procedures. Applicants respectfully disagree. Applicants point out that the experiments in the specification do, in fact, provide evidence that a broad range of genes can be activated.

The Examiner is correct that only integral membrane proteins were detected in the experiments. Applicants point out, however, that these experiments were specifically designed to detect only activated integral membrane proteins; only cells expressing an activated integral membrane protein were selected. Cells not expressing an activated integral membrane protein were removed during the selection procedure. The rationale for the procedure is explained on page 74 of the Applicants' specification and is also addressed in the attached Declaration (see below).

Accordingly, in an experiment designed to detect <u>only</u> activated integral membrane proteins, activation of integral membrane proteins was achieved. It is reasonably predictable, therefore, that cells expressing any given activated gene could be identified by using an identification procedure specific for that given activated gene. Examples of assays to screen for the activation of other types of genes are provided in Applicants' specification. For example, see pages 65-68.

The above argument notwithstanding, Applicants assert that they provided the person of ordinary skill in the art with vectors, procedures for using these vectors, and procedures to activate a broad range of endogenous genes. Accordingly, the Applicants present the attached Declaration of Dr. John J. Harrington, an inventor of the claimed subject matter, showing that (at the time of filing) the invention could have been made and used by the person of ordinary skill in the relevant arts, as disclosed in the specification, by routine and ordinary experimental techniques.

Applicants summarize the points made in the Declaration as follows:

(1) Applicants have activated a wide range of genes using the Applicants' specification as a guide and by means of routine experimentation available to the person of ordinary skill in the art at the time of filing.

(2) Many of the activated genes (around 20,000 genes, most of which are estimated to be unique) are on the claimed list of genes.

(3) Whenever Applicants have assayed for activated genes to ascertain if a specific gene of interest had been activated, they have found activation of the gene of interest. Such genes include genes on the list and many other unlisted genes of interest.

(4) The experiments in the Examples show activation of integral membrane proteins and not other types because the experiments were designed to detect only this type of protein. Cells expressing other types of activated genes were not selected for; they would have been removed according to the experimental design.

(5) Activation (increased expression) has been shown to occur relative to the parental cell line. Activation is associated with integration of the activation constructs.

(6) Accordingly, the probability of activating any given gene is <u>not zero</u>.

(7) Applicants have achieved faithful expression of activated gene products based on physical and biochemical criteria.

(8) It is reasonably predictable that activated gene expression would occur in cells introduced *in vivo* for the same reasons that prior art recombinant genes are expressed in cells introduced into an animal.

(9) A variety of screening procedures is available to assay for activation of a given gene of interest.

On the basis of the evidence and discussion above and in the Declaration, Applicants submit that all grounds of rejection under 35 U.S.C. §112, first paragraph, have been duly addressed. Reconsideration and withdrawal of this rejection is therefore respectfully requested.

B. The Rejection Under 35 U.S.C. § 112, Second Paragraph

On page 5 of the Office Action, claims 64-69, 71-74, 76-82, 85-132, 159-162, 164-175, and 177-183 have been rejected under 35 U.S.C. § 112, second paragraph, on the grounds that they are indefinite. Applicants respectfully traverse the rejection.

Claims 64-69, 71-74, 76-82, 104-108, 128-132, 159-162, 164-175, and 177-183 are rejected for being dependent on a non-elected invention. Applicant points out, however, that the Examiner included these claims in Group VII, which is the elected group in this application. See the Restriction Requirement dated September 27, 1999, page 2, Group VII. Accordingly, it appears that either antecedent claims should be included in this group or that the subject claims were included in this group in error. Applicant respectfully requests clarification.

Claim 82 "and dependent claims" are rejected because "said gene product" lacks "antecedent basis." First, Applicants are not clear what claims constitute "and dependent claims." Applicants ordinarily would understand this phrase to refer to claims that depend from claim 82. However, none of the subsequent claims depend from claim 82. Applicants

accordingly request clarification. With respect to claim 82, antecedent basis for "said gene product" occurs in line 1 ("a gene product").

Claims 77 and 109-112 "and dependent claims" are rejected on the grounds that the term "genome-containing cell" is unclear. Applicants respectfully submit that the term "genome" is used in its ordinary sense. Nevertheless, Applicants have eliminated this term from the claim, as they believe that the term is not necessary to define the claim.

Claims 130-132 are rejected because they depend from claim 89 that "does not recite an *in vivo* method." Claim 130 has been canceled. Accordingly, the point is moot.

Claim 82 has been rejected on the grounds that it does not recite "necessary steps," such as "steps of screening" or "method of screening." Applicants point out that the claim contains several steps. These include transfecting cells with a construct, allowing integration of the construct into genomic DNA, screening the cells for gene expression, selecting expressor cells, and obtaining the gene product from the selected cell. Thus, the claim as a whole is directed to a method for obtaining a gene product. Screening is only one of the steps. The claim is not specifically directed to methods for screening and therefore, § 112, second paragraph, does not require that specific screening methods be recited. To do so would be to unduly limit the claim. Applicants note that the Examiner has not indicated that a specific method for selection is required to render the claim definite. On the same principle, no specific method of screening does not render the claim indefinite. § 112, second paragraph, requires that the metes and bounds of a claim be defined so that the public would be clear on the subject matter that constitutes infringing subject matter. Applicants submit that the public would know if it infringed the claim whether or not a specific screening method is set forth. If they practiced all of the claimed steps, exactly as recited, using all of the claimed compositions, they would know that the claim is infringed (*i.e.* using <u>any</u> screening method). Accordingly, Applicants submit that the claim is definite.

Claims 85-87, 98, 109, 113, and 116 are similarly rejected on the grounds that they do not recite all the "necessary steps" of the method, such as "method of screening." Applicants

respectfully submit that screening in these claims is but one of several steps recited in these method claims. These claims are directed to methods for producing or overexpressing an expression product, not to methods of screening. For the reasons noted in the preceding paragraph, Applicants submit that these claims are also definite.

Claim 165 has been rejected for the recitation of "genomic DNA." The Examiner asks "genomic DNA of what?" Applicants point out that the term is discussed in the specification. Please see pages 104, line 4 to 105, line 21. Accordingly, Applicants believe that the claim is definite in the recitation of "genomic DNA."

In view of the above amendments and discussion, Applicants submit that all grounds of rejection under 35 U.S.C. §112, second paragraph, have been addressed. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

C. The Rejection Under 35 U.S.C. § 102(b)

On page 6 of the Office Action claims 58, 59, 64, 65, 69, 74, 76, and 225 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Duyk *et al.* (*Proc. Nat'l Acad. Sci. U.S.A.* 87: 8995-8999 (1990)), herein "Duyk." Applicants respectfully traverse the rejection.

Duyk teaches a plasmid vector with the following elements: LTR; unpaired splice donor; α-complementing factor of *E. coli* β-galactosidase (α-β-gal); SV40 origin of replication and early promoter; aminoglycoside phosphoribosyltransferase (neo) gene from *E. coli* Tn5; ColE1 origin of replication; CAT gene of *E. coli* Tn9; retrovirus packaging sequences; human β-globin exon I. See Figure 1.

The purpose of the Duyk vector is to identify exons in genomic DNA cloned into the vector. The strategy is to recover exons from random pieces of genomic DNA that are inserted in the vector by shotgun cloning. The insertion site is downstream from an "exon trap" that consists of a functional splice donor and an intervening sequence incorporating the α-β-gal gene followed by a multiple cloning site. The DNA is transcribed and transcripts derived from the cloned

genomic DNA containing a splice acceptor undergo a splicing event that results in loss of the marked intervening sequence from the vector. The loss of the marked sequence allows selection of vectors containing DNA derived from RNA that has undergone a splicing event.

The Duyk vector does not anticipate the Applicants' claims. The Applicants' claimed vectors contain one or more amplifiable markers. The Duyk vector lacks an amplifiable marker. Therefore, the reference does not anticipate the claims.

Furthermore, Duyk provides no suggestion or motivation to include an amplifiable marker. The purpose of the α-β-gal marker is to allow detection of a splicing event. This marker adequately serves this purpose. Therefore, there would have been no reason to substitute an amplifiable marker. Likewise, the neo gene provides for detection of recombinant (*i.e.*, plasmid-containing) cells and is adequate for this intended purpose. Applicants also point out that there would have been no reason to amplify the genomic sequence on the vector. The splicing event is detected by means of the β-gal marker sequence, not by expression levels of gene products from the genomic DNA.

In fact, the use of an amplifiable marker on the Duyk vector would be counterproductive to the goal of the Duyk methods. Inclusion of an amplifiable marker, and subsequent use of the amplifiable marker, would interfere with the intended use of the Duyk vector. For example, if an amplifiable marker were included on the vector, and cells containing the integrated vector were subjected to selection for amplified copies of the integrated vector, it would not be possible to obtain a representative sample of trapped exons. One reason for this is that some genetic loci amplify more efficiently. As a result, in a population of cells containing integrated vectors, some trapped exons (derived from the exogenous genomic DNA cloned into the vector prior to transfection) would be preferentially amplified, while other exons would either not amplify or would amplify to a lesser extent. Thus, it would be difficult to recover all of the trapped exons due to the biases introduced during amplification. Therefore, inclusion of and selection for an amplifiable marker would interfere with the process set forth by Duyk.

Accordingly, Duyk neither discloses nor suggests a vector with a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence and one or more amplifiable markers. As such, this reference neither anticipates nor suggests the Applicants' claimed invention.

In view of this discussion, Applicants submit that all grounds of rejection under 35 U.S.C. §102(b) have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

D. The Rejection Under 35 U.S.C. § 102(e)

On page 7 of the Office Action, claims 94-97 and 107 have been rejected under 35 U.S.C. § 102(e) as being anticipated by or obvious over U.S. 5,688, 679 ("the '679 patent"). Applicants respectfully traverse the rejection.

The '679 patent discloses recombinant expression of an Apa I restriction fragment of the human erythropoietin gene (Epo). Host cells include COS-7 and BHK cells.

Claims 96 and 97 are directed to growth hormone and granulocyte-colony stimulating factor, respectively. The '679 patent is specifically limited to Epo. Therefore, this patent neither anticipates nor suggests these proteins.

Applicants point out that it is possible to produce Epo (and other proteins) that is distinguishable over the Epo proteins produced in COS-7 and BHK cell lines disclosed in the '679 patent, using the claimed processes. Nevertheless, Applicants have deleted claims 94 and 95 without prejudice or disclaimer to expedite prosecution, and reserve the right to file one or more patent applications directed to such distinguishable proteins.

In view of this discussion, Applicants submit that all grounds of rejection under 35 U.S.C. §102(e) have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

E. The Rejections Under 35 U.S.C. § 103(a)

1. The Rejection Over Duyk in View of Kaufman

On page 8 of the Office Action, claims 66-68 and 71-72 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Duyk in view of Kaufman (*Short Protocols In Molecular Biology*, pp. 16-58) (1995), herein "Kaufman"). Applicants respectfully traverse the rejection.

Duyk is relied upon as above. The Examiner indicates, however, that this reference does not teach a CMV, non-viral, or inducible promoter. The Examiner also indicates that the reference does not teach vectors integrated into the genome of a cell. Accordingly, the Examiner relies upon Kaufman for these elements. The Examiner then concludes that it would have been obvious to modify the vector of Duyk by using different promoters, such as CMV or an inducible promoter. The rationale is that there would have been motivation to use such a promoter because the expression level of foreign genes under the control of different promoters varies depending on the cell type. The Examiner also indicates that the CMV promoter is simply another commonly used promoter for recombinant expression. Applicants respectfully disagree.

The Duyk construct is a retrovirus construct. The experimental strategy used by Duyk depends on the production of retrovirus (see the numbered protocol below). The strategy is designed to produce infectious retrovirus that can be packaged, used to infect, is capable of integration and of being transcribed as a provirus and reverse transcribed as RNA. The LTRs are crucial, therefore, to carry out these functions. To substitute another promoter, as the Examiner has proposed, would destroy the retrovirus function. Therefore, it would destroy the experimental design used by Duyk. See Duyk, page 8996, "Experimental Strategy." Since the LTR would have to be retained, the person of ordinary skill in the art would not have been motivated to substitute another promoter, as proposed.

For the Examiner's convenience, Applicants outline the steps in the experimental protocol used by Duyk.

(1) Transfect a packaging cell line with pETV-SD containing a genomic sequence cloned in the MCS;

(2) allow the vector to integrate;

(3) allow RNA transcription from the integrated vector beginning in the U3 part of the left LTR and terminating in the U5 part of the right LTR, to produce retroviral RNA;

(4) allow splicing from the splice donor on the vector to a splice acceptor (if one is present in the cloned genomic DNA);

(5) select, using G418, cells in which the vector has integrated (neo gene provides positive selection in the presence of G418);

(6) allow packaging of the retrovirus RNA in the selected cells to produce infectious virions;

(7) infect PA-317 cells with the infectious virus to increase the probability of splicing;

(8) select with G418 to select for cells expressing the virus (*i.e.*, neo);

(9) infect COS cells with the retrovirus of step (8);

(10) allow production of episomal DNA in the COS cells;

(11) transform *E. coli* with the episomal DNA of step (10);

(12) select for cells that lack the α-β gal sequence (spliced out in step (4)) using IPTG.

Applicants also point out that Duyk, the primary reference, cannot be relied upon for the reasons discussed above. Claims 66-68 and claims 71-72 are ultimately dependent upon claims 58-59. As discussed above, claims 58-59 are directed to vectors containing amplifiable markers. Duyk does not disclose an amplifiable marker. Further, as noted, there would have been no

reason to modify the Duyk vector to include such a marker since the α-β-gal marker in the vector was adequate for its intended purpose.

In view of this discussion, Applicants submit that this ground of rejection under 35 U.S.C. §103(a) has been addressed and the rejection overcome. Reconsideration and withdrawal of this rejection is therefore respectfully requested.

2. The Rejection Over Duyk in View of Anderson (U.S. 5,629,159)

On Page 9 of the Office Action, claims 157, 160, 161, 162, 164, 166, 168-170, 172-174, 177, and 178 have been rejected under 37 U.S.C. § 103(a) as being obvious over Duyk in view of U.S. 5,629,159 ("the '159 patent"). Applicants respectfully traverse this rejection.

The Examiner relies on Duyk as summarized above. The Examiner states that Duyk teaches a vector containing multiple promoters, reporter genes, selectable markers, unpaired splice donor site, and origins of replication, but not a negative selection marker under the regulation of a promoter. The Examiner relies on the '159 patent to teach this aspect.

First, Applicants point out that claims 160, 161, 162, 164, 166, 168-170, 172-174, 177, and 178 are dependent upon claim 157. Claim 157 states:

A vector construct comprising:

(a) a first promoter operably linked to a positive selectable marker;

(b) a second promoter operably linked to a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said positive and negative selectable marker and said splice donor site are oriented in said vector construct in an orientation that, when said vector is integrated into the genome of a eukaryotic host cell in such a way that an endogenous gene in said genome is transcriptionally activated, then said positive selectable marker is expressed in active form and said negative selectable marker is either not expressed or is expressed in inactive form.

Applicants have described multiple vector configurations, disclosed, for example, in Figures 10A-F, that have these characteristics. The negative selectable marker will always be configured, relative to the splice donor, such that upon splicing of the splice donor onto a splice acceptor, the negative selectable marker will be lost or inactivated.

The Examiner states that it would have been obvious to modify the Duyk vector by replacing the neo gene with a negative selection marker, linearize the vector at the MCS, and integrate this vector into a cellular genome. Applicants respectfully disagree for several reasons.

First, replacing the neo gene with a negative selectable marker would destroy the Duyk experimental strategy. The neo gene is necessary to select for cells containing the retrovirus vector (see steps (5) and (8) above). If this gene were to be replaced with a marker that killed cells expressing it, no virus-producing cells would be recovered. Only the parental (non-recombinant) cells or grossly rearranged cells would survive. Accordingly, the person of ordinary skill in the art would not have been motivated to make the proposed replacement.

Second, linearizing the Duyk vector at the cloning site would also destroy the experimental strategy. According to Duyk, the integrated vector used to recover splice events is one in which a genomic sequence is present. The function of the vector would be destroyed because linearizing the vector at the cloning site would separate the splice donor sequence from the cloned genomic DNA. The purpose of the vector is to identify exons in the cloned genomic DNA by allowing a splicing event between the splice donor on the vector and an exon in the cloned genomic DNA. If these two components are separated, no exons could be identified because no such splicing event could occur. Therefore, the person of ordinary skill in the art would not have been motivated to linearize the vector at the cloning site prior to integration.

Moreover, linearizing at the cloning site would destroy the experimental strategy in that functional retrovirus would not be produced after the first integration step (step 2, above). Integration of the linearized vector would produce LTRs that would no longer be configured so as to produce functional retrovirus: the 5' LTR would be downstream from the 3' LTR. The packaging sequence (as well as the IVS, splice donor and α-β gal) would be downstream from

the second integrated LTR (originally the 5' LTR). Packaged transcripts would lack a 3' LTR and therefore could not produce functional retrovirus. (The genomic DNA, SV40 ori, neo gene, and ColE1 ori would be upstream from the first integrated LTR (originally the 3' LTR) and therefore would not be transcribed from either LTR.)

The Examiner further states that β-gal would be expressed because it was joined to a splice acceptor site in the exon in the genomic DNA, so selection could be with β-gal or a positive selection marker. Applicants point out, however, that the presence of a splice acceptor would <u>delete</u> the β-gal gene. Therefore, it wouldn't be expressed at all. In fact, loss of this gene is the key to the screening procedure for a splicing event.

The Examiner also refers to two populations of cells, but only discusses one of these, *i.e.*, "cells will be expressing both beta-galactosidase and the negative selection gene." As to this one, however, Applicants have already discussed why the person of ordinary skill in the art would not have been motivated to modify the Duyk vector with a negative selectable marker in place of neo and why β-gal would <u>not</u> be expressed if the genomic DNA contained a splice acceptor.

The above argument addresses the substantive issue regarding a lack of motivation to combine references. Applicants point out, however, that, in any event, Duyk is improperly relied upon for the reasons discussed above (no disclosure or suggestion to modify the Duyk vector by adding an amplifiable marker). Further, even if the motivation to combine references did exist, this combination would not produce the claimed invention because none of the references discloses or suggests modifying the Duyk vector by adding an amplifiable marker.

In view of this discussion, Applicants submit that all grounds of rejection have been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

3. The Rejection Over Duyk and Anderson and Further in View of U.S. 5,677,170

On Page 11 of the Office Action, claims 159, 167, and 178 have been rejected under 37 U.S.C. § 103(a) for being obvious over Duyk and the '159 patent and further in view of U.S. 5,677,170 ("the '170 patent"). Applicants respectfully traverse the rejection.

The Examiner relies upon Duyk and the '159 patent as discussed above. He indicates, however, that these references do not disclose or suggest transposition signals in the disclosed vectors. Accordingly, the Examiner relies upon U.S. 5,677,170 as teaching artificial transposons to introduce DNA elements into plasmid targets. The Examiner then concludes that it would have been obvious to modify the Duyk vector to include transposition sequences.

The Applicants point out that claim 159 recites the vector of claim 157 and claims 58-59 further containing one or more transposition signals.

Applicants respectfully assert that there would have been no motivation to modify the Duyk vector with transposition sequences. As discussed above, the Duyk vector was fully functional for its intended purpose, exon trapping. The Duyk vector was designed so that genomic DNA could be cloned into the vector. There was no need to include transposition signals on the vector for the purpose of introducing genomic DNA into the vector. Furthermore, the vector containing a genomic DNA insert was introduced into a host cell, allowing splicing events to be recovered and detected. Including transposition signals on the vector would not aid in this process. The vectors and methods described by Duyk were capable of carrying out the intended function, that is, recovering exons (*i.e.*, splicing products).

The Examiner also suggests that "it would have been obvious to an artisan of ordinary skill in the art to modify the vector of Duyk *et al.* by including transposition sequences of Devine *et al.* with reasonable expectation of success, linearize the vector, transfect in a cell, and select the cells that would have expressed beta-gal but not the negative selection marker because Duyk *et al.*, Anderson, and Devine *et al.* teach all the necessary methods and sequences. An artisan would have been motivated to include the transposon in the vector, create a library of cells that

comprise the vector because due to the random integration of the vector in the genome of cells, *cells would have integrated the vector in different exons* and thus trap vectors that could then be isolated." Italics added.

Applicants respectfully disagree. First, none of the references suggest integrating the vector into different (endogenous) exons. Duyk describes a method for identifying exons present in cloned genomic fragments. Devine describes a method of *in vitro* (but not *in vivo*) transposition. Anderson describes a vector and methods for expressing foreign (*i.e.*, exogenous) immortalization genes. Thus, there is no support in any of the cited references for integrating the vector into endogenous exons.

Second, there is no reason to include transposition sequences on the vector in the hypothetical method outlined by the Examiner. The vectors, following linearization, will integrate into the host cell genome without any transposition sequences. Furthermore, without transposase, even if transposon signals were present on the vector, they would not facilitate integration *in vivo*. Again, the Applicants point out that all methods described by Devine involve *in vitro* transposition, not *in vivo* transposition.

Third, β-gal in the Duyk vector is expressed from bacterial promoter and translation elements. As a result, β-gal can not be expressed in eukaryotic host cells. Thus, without β-gal expression, it is not possible to select cells expressing β-gal, nor is it possible to identify cells in which an endogenous exon has been trapped, as suggested in the Examiner's hypothetical method.

Fourth, as discussed above, the negative selection marker described by Anderson, unlike the negative selectable marker of the present invention, would be expressed regardless of whether an endogenous exon has been trapped. As a result, in the hypothetical method suggested by the Examiner, every cell receiving an integrated copy of the vector (containing the Anderson selectable marker) would die during selection, and therefore, no endogenous exons could be activated or isolated.

Further, the '170 patent discusses the use of artificial transposons for DNA sequencing and for introducing marker genes into DNA. In fact, Devine lists a number of uses for *in vitro* transposition, none of which involve combining transposon vectors with vectors containing promoter/exon units such as those disclosed in the present application; and the general statement made by Devine that "these (vectors) can be used to introduce any functional or non-functional DNA cis elements, sequences, or combination into another segment of DNA" does not constitute an enabling disclosure that would guide one of skill in the art specifically to choose exon trapping vectors to combine with the transposon. Indeed, exon trapping vectors are fully capable of carrying out their intended use. Thus, there is no disclosure or suggestion to convert the highly specific and complex Duyk vector to an artificial transposon for exon identification or any other use.

Thus, it is not obvious to combine the cited references. Furthermore, if the references are combined in the manner proposed by the Examiner, for the reasons detailed above, the vectors will not be useful.

Applicants further point out that both exon trapping techniques and *in vitro* transposition reactions have been used for over 10 years. Despite this extensive period of time, no one has ever suggested combining the two approaches. The fact that no one from either of these two well-developed fields has ever suggested combining the two approaches demonstrates that it was not obvious to do so.

For the above reasons alone, Applicants assert that the person of ordinary skill in the art would not have been motivated to combine Duyk with the '159 and '170 patents to produce the Duyk vector with transposition sequences.

Without further addressing the substantive argument regarding motivation to combine references, Applicants point out that, in any event, Duyk is improperly relied upon for the reasons discussed above (no disclosure or suggestion to modify the Duyk vector by adding an amplifiable marker). Further, even if a motivation to combine references did exist, this

combination would not produce the claimed invention because none of the references discloses or suggests modifying the Duyk vector by adding an amplifiable marker.

In view of this discussion, Applicants submit that this grounds of rejection under 35 U.S.C. §103(a) has been addressed and the rejection overcome. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

CONCLUSION

Accordingly, in view of the above remarks and amendments, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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ALSTON & BIRD LLP P.O. Drawer 34009 Charlotte, NC 28234 Tel Raleigh Office (919) 420-2200 Fax Raleigh Office (919) 420-2260	**CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner For Patents, Washington, DC 20231, on April 24, 2000. Nora C Martinez Nora Martinez

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